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(FILE 'HOME' ENTERED AT 07:41:53 ON 06 APR 2003)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 07:42:42  
ON 06 APR 2003

L1 15040 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P)  
(NONSPEC  
L2 77051 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (CELL?)  
L3 11419 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (STAIN?)  
L4 150 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (TYRAMID  
L5 3696 S L1 (6P) L2  
L6 420 S L5 (6P) L3  
L7 420 S L5 (6P) L6  
L8 420 S L5 (3P) L6  
L9 5 S L1 (3P) L2 (3P) L3 (3P) L4  
L10 2 DUP REM L9 (3 DUPLICATES REMOVED)  
L11 5 S L1 (6P) L2 (6P) L3 (6P) L4  
L12 344 DUP REM L8 (76 DUPLICATES REMOVED)  
L13 1 S L12 (6P) (CHAOTROP? (6A) WASH?)  
L14 540 S CHAOTRO?/TI  
L15 1 S L12 AND L14  
L16 6203 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (NONSPECI  
L17 5432 S L2 (3P) L3  
L18 143 S L17 (3P) L16  
L19 4 S L18 (3P) L4  
L20 4 S L18 AND L4  
L21 2 DUP REM L20 (2 DUPLICATES REMOVED)  
L22 6 S L18 AND (?TYRAMINE? OR ?TYRAMIDE?)  
L23 4 DUP REM L22 (2 DUPLICATES REMOVED)  
L24 143 S L17 (P) L16  
L25 85 DUP REM L24 (58 DUPLICATES REMOVED)  
L26 17 S L4 (3P) (DENATUR?)  
L27 11 DUP REM L26 (6 DUPLICATES REMOVED)  
L28 38 S L6 (6P) (DENATUR? (P) CELL?)  
L29 38 DUP REM L28 (0 DUPLICATES REMOVED)  
L30 9872 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3A)  
(DENATUR  
L31 56002 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (CELL?)  
L32 1826 S L30 (P) L31  
L33 2 S L32 (6P) (?TYRAMI?)  
L34 16 S L32 (6P) L16  
L35 8 DUP REM L34 (8 DUPLICATES REMOVED)  
L36 1 S L32 (6P) ((NONSPECIFIC OR NON-SPECIFIC) (3A) (STAIN? OR BACK  
L37 495 S L32 (6P) (STAIN? OR BACKGROUND OR SIGNAL?)  
L38 35 S L32 (6P) (NONSPECIFIC OR NON-SPECIFIC)  
L39 15 S L37 AND L38  
L40 15 DUP REM L39 (0 DUPLICATES REMOVED)  
L41 5 S L32 (6P) (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L42 41 S L32 AND (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L43 7 S L32 (12P) (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L44 7 DUP REM L43 (0 DUPLICATES REMOVED)  
L45 106 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3A) (DENATUR  
L46 69 DUP REM L45 (37 DUPLICATES REMOVED)  
L47 64 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (SIGNAL?  
L48 0 S L47 AND L45  
L49 41 DUP REM L47 (23 DUPLICATES REMOVED)

ACCESSION NUMBER: 95326610 MEDLINE  
DOCUMENT NUMBER: 95326610 PubMed ID: 7541493  
TITLE: ImmunoMax. A maximized immunohistochemical method for the  
retrieval and enhancement of hidden antigens.  
AUTHOR: Merz H; Malisius R; Mannweiler S; Zhou R; Hartmann W;  
Orscheschek K; Moubayed P; Feller A C  
CORPORATE SOURCE: Department of Pathology, Medical University of Lubeck,  
Germany.  
SOURCE: LABORATORY INVESTIGATION, (1995 Jul) 73 (1) 149-56.  
Journal code: 0376617. ISSN: 0023-6837.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199508  
ENTRY DATE: Entered STN: 19950822  
Last Updated on STN: 19960129  
Entered Medline: 19950810

**AB BACKGROUND:** Since the introduction of mAb, immunohistochemistry has become an important tool in research and in surgical pathology. The most widely used fixative in routine histopathology is formaldehyde, and it has become the gold standard for morphologic tissue preservation. Although the molecular mechanism underlying the tissue fixation is not well understood, it has become clear that available immunoreactive Ag are progressively lost during the fixation process. For a long time, it was thought that formalin-sensitive Ag might be irreversibly destroyed during the fixation process. Although monoclonal anti-Ig Ab frequently worked inadequately, polyclonal anti-Ig Ab were shown to produce reproducible staining results. It thus appeared possible that most cellular Ag might not be irreversibly destroyed but only masked. **EXPERIMENTAL DESIGN:** Although some Ag may be retrieved under appropriate conditions, there might still be many for which available antigenic epitopes are still too sparse to be visualized, as observed for a large number of leukocyte differentiation Ag. One reliable approach to resolve this dilemma is the use of a combination of an optimized Ag retrieval system and a powerful immunohistochemical staining protocol introducing a biotin amplification step, in which signal amplification is accomplished by covalent deposition of biotin molecules. **RESULTS:** Cryostat and paraffin sections were stained with the avidin-biotin complex technique and, for comparison, with the new maximized immunohistochemical staining protocol, termed the ImmunoMax method. Each step was monitored to establish how effectively it enhanced the overall sensitivity. Although pretreatment with detergent, protease, a chaotropic substance, or microwave heating resulted in only moderately improved immunostaining, the biotinylated tyramine enhancement step proved to be the most efficient one, although the latter is not sufficient for many Ag when used without pretreatment steps. The combination of an Ag retrieval step with the biotinylated tyramine enhancement step resulted in a 100 to 10,000-fold boost in sensitivity without loss of specificity. **CONCLUSIONS:** With the ImmunoMax method, defined Ag can be reproducibly detected in formalin-fixed, paraffin-embedded tissues, and the sensitivity of the method is tremendously enhanced. Moreover, it also allows many previously unreactive or unsatisfactorily reactive Ag to be detected, as shown here for IgD, IgM, and CD7 with the use of mAb.

ACCESSION NUMBER: 2002:325978 USPATFULL  
TITLE: Use of a1b1 integrin receptor inhibitors and

TGF-b1 inhibitors in the treatment of kidney  
disease

INVENTOR(S): Cosgrove, Dominic, Omaha, NE, United States

PATENT ASSIGNEE(S): Boys Town National Research Hospital, Omaha, NE, United  
States (U.S. corporation)

NUMBER KIND DATE

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PATENT INFORMATION: US 6492325 B1 20021210  
APPLICATION INFO.: US 1999-292534 19990415 (9)  
RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-150485, filed  
on 9 Sep 1998, now abandoned Continuation-in-part of  
Ser. No. US 1998-88766, filed on 2 Jun 1998, now  
abandoned

NUMBER DATE

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PRIORITY INFORMATION: US 1998-86587P 19980522 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Borin, Michael

LEGAL REPRESENTATIVE: Muetting, Raasch & Gebhardt, P.A.

NUMBER OF CLAIMS: 16

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 24 Drawing Figure(s); 24 Drawing Page(s)

LINE COUNT: 2733

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods for treating (i.e., delaying the  
onset of, slowing the progression of, and/or reversing) kidney disorders  
(e.g., renal glomerulonephritis and/or renal fibrosis). Certain of these  
methods involve administering an  $\alpha 1\beta 1$  integrin receptor  
inhibitor optionally in combination with a TGF-b1 inhibitor. The  
present invention also provides a mouse model for kidney disease wherein  
the mouse does not express a normal collagen type 4 composition in the  
GBM (i.e., it does not incorporate collagen  $\alpha 3(IV)$ ,  $\alpha 4(IV)$ ,  
and  $\alpha 5(IV)$  chains into its glomerular basement membrane) and does  
not express the  $\alpha 1\beta 1$  integrin receptor.

DETD . . . Slides were fixed for 15 minutes by soaking either in cold  
(-20° C.) 95% ethanol, if to be used for staining with the  
basement membrane collagen-specific antibodies, or with cold (20°  
C.) acetone for staining with antibodies specific for the basement  
membrane associated proteins. Slides were allowed to air dry overnight,  
and stored desiccated at. . .

DETD Samples were allowed to reach ambient temperature, then washed three  
times in PBS (pH 7.4) at room temperature. For staining with the  
antibodies against the type IV collagens, the tissue was pretreated with  
0.1M glycine and 6M urea (pH 3.5) to denature the protein and expose  
the antigenic sites. The appropriate dilutions (determined empirically)  
of the primary antibodies. . . into a solution of 5% nonfat dry milk  
in PBS (pH 7.4). The use of nonfat dry milk substantially reduced  
background fluorescence. Samples were washed four times in PBS (pH  
7.4) for 10 minutes each at room temperature to remove the. . .  
Samples were sealed under glass cover slips using clear nail polish.  
Slides were photographed at 1000 $\times$  magnification. Jones silver  
methenamine staining was performed on plastic embedded specimen.

DETD . . . purchased from Southern Biotechnology, Inc., Birmingham, Ala.  
This antibody was tested for cross reactivity by the manufacturer, and  
produced a staining pattern in the glomerulus that is consistent with

that observed for other antibody preparations against these chains (Miner and Sanes, J. Cell. Biol., 127:879-891, 1994). Anti-heparin sulfate proteoglycan (HSPG) antibody is a rat monoclonal raised against the HSPG core protein purified from. . . as for the absence of cross-reactivity with other major basement membrane components by western blot analysis (Ljubimov et al., Exp. Cell Res., 165:530-540, 1986).

DETD Immunofluorescence and Jones stained images were recorded and processed using an Olympus BH2 RFLA fluorescence microscope interfaced with an Applied Imaging Cytovision Ultra image. . .

DETD . . . rabbit anti-mouse, and was purchased from Biogenesis, Inc. (Sandown, N.H.). The antisera was used at a 1:100 dilution for immunoperoxidase staining. The fibronectin antibody used was the same as for immunofluorescence staining (a rabbit anti-human fibronectin antisera from Sigma Chemical Company, St. Louis, Mo.), and was used at a 1:100 dilution. Secondary. . .

L40 ANSWER 13 OF 15 USPATFULL

ACCESSION NUMBER: 1998:4404 USPATFULL

TITLE: Manual in situ hybridization assay

INVENTOR(S): Bresser, Joel, Bellaire, TX, United States

Evinger-Hodges, Mary Jean, Arlington, TX, United States

PATENT ASSIGNEE(S): Aprogenex, Inc., Houston, TX, United States (U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 5707801 19980113

APPLICATION INFO.: US 1995-421705 19950413 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1991-668751, filed on 13 Mar 1991, now abandoned which is a continuation of Ser. No. US 1988-239491, filed on 31 Aug 1988, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Jones, W. Gary

ASSISTANT EXAMINER: Rees, Dianne

LEGAL REPRESENTATIVE: Elman & Associates

NUMBER OF CLAIMS: 57

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 22 Drawing Figure(s); 14 Drawing Page(s)

LINE COUNT: 1786

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A rapid, sensitive in situ hybridization assay is provided which will detect as few as 1-5 copies of target biopolymer per cell and may be accomplished in 2-4 hours. There is provided a quantitative assay which may be used to diagnose and monitor treatment of diseases.

SUMM The invention of the present application which provides optimal fixatives allowing probe entry and blocking of non-specific probe binding and formamide hybridization at high temperatures (55° C.) provides a hybridization assay with rapid kinetics of hybrid formation.

SUMM The hybridization solution consists of a chaotropic denaturing agent, a buffer, a pore forming agent, a hybrid stabilizing agent, non-specific nucleotides, and a target specific probe.

SUMM The chaotropic denaturing agent (Robinson, D. W. and Grant, M. E.

(1966) J. Biol. Chem. 241:4030; Hamaguchi, K. and Geiduscheck, E. P. (1962) J. Am. Chem. Soc. 84:1329) is selected from the group consisting of formamide, urea, thiocyanate, guanidine, trichloroacetate, tetramethylamine, perchlorate, and sodium iodide. Any buffer which maintains pH at least between 7.0 and 8.0 may be. . . location of the target biopolymer, the pore-forming agent is chosen to facilitate probe entry through plasma, or nuclear membranes or cellular compartmental structures. For instance, 0.05% 23-Lauryl-ether or 0.1% Octyl-phenoxy-polyethoxy-ethanol will permit probe entry through the plasma membrane but not the. . . biopolymer probe may also be selected such that its size is sufficiently small to traverse the plasma membrane of a cell but is too large to pass through the nuclear membrane.

SUMM In order to prevent non-specific binding of nucleic acid probes, nucleic acids unrelated to the target biopolymers are added to the hybridization solution at a. . .

SUMM In addition, cellular nucleic acids were stained with about 50 mg/ml propidium iodide dye. This dye has a specific characteristic fluorescent emission (about 480 nm, green) when. . .

L44 ANSWER 3 OF 7 USPATFULL

ACCESSION NUMBER: 2002:175291 USPATFULL

TITLE: Replicable hybridizable recombinant RNA probes and methods of using same

INVENTOR(S): Kramer, Fred Russell, Riverdale, NY, United States  
Lizardi, Paul M., Cuernavaca, MEXICO  
Miele, Eleanor Ann, Brooklyn, NY, United States  
Mills, Donald R., Engelwood, NJ, United States

PATENT ASSIGNEE(S): The Trustees of Columbia University in the City of New York, New York, NY, United States (U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 6420539 B1 20020716

APPLICATION INFO.: US 1995-484992 19950607 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-296866, filed on 26 Aug 1994, now patented, Pat. No. US 5503979, issued on 2 Apr 1996 Continuation of Ser. No. US 1993-118476, filed on 8 Sep 1993, now abandoned Continuation of Ser. No. US 1992-988356, filed on 9 Dec 1992, now abandoned Continuation of Ser. No. US 1990-527585, filed on 23 May 1990, now abandoned Continuation-in-part of Ser. No. US 1988-183838, filed on 20 Apr 1988, now abandoned Continuation-in-part of Ser. No. US 1986-852692, filed on 16 Apr 1986, now patented, Pat. No. US 4957858, issued on 18 Sep 1990 Continuation-in-part of Ser. No. US 1984-614350, filed on 25 May 1984, now patented, Pat. No. US 4786600, issued on 22 Nov 1988

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Horlick, Kenneth R.

ASSISTANT EXAMINER: Tung, J.

LEGAL REPRESENTATIVE: White, John P., Cooper & Dunham LLP

NUMBER OF CLAIMS: 24

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 13 Drawing Figure(s); 13 Drawing Page(s)

LINE COUNT: 2298

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a replicatable and hybridizable recombinant single-stranded RNA probe molecule comprising: a recognition sequence for the binding of an RNA-directed RNA polymerase; a sequence required for the initiation of product strand synthesis by the polymerase; and a heteroloqus RNA sequence inserted at a specific site in the internal region of the recombinant molecule and complementary to an oligo or polynucleotide of interest. This invention also provides methods for determining the presence or concentration of an oligo- or polynucleotide of interest in a sample and for simultaneously determining the presence or concentration of several different oligo- or polynucleotides of interest in a sample.

SUMM . . . screen a large number of samples, the selected format has to be fast and simple, thus precluding the fractionation of cells or the isolation of nucleic acids, and necessitating the use of solution hybridization; and (b) because nonhybridized probes are amplified. . . format must include an extremely efficient means of removing the nonhybridized probes. Hybridization is extremely efficient in solutions of the chaotropic salt, guanidine thiocyanate (Thompson and Gillespie, 1987), and concentrated solutions of guanidine thiocyanate will lyse cells, denature all proteins (including nucleases), liberate nucleic acids from cellular matrices, and unwind DNA molecules, permitting hybridization to occur without interference from cellular debris (Pelligrino, et al. 1987). The "reversible target capture" procedure (Morrisey, et al. 1989) is an efficient means for removing. . .

SUMM . . . achieved. Moreover, probes that are not bound to their targets cannot be elongated, so their presence does not generate a background signal. However, there are a number of significant disadvantages to using the polymerase chain reaction: DNA polymerase is inhibited by many. . .

L46 ANSWER 11 OF 69 USPATFULL

ACCESSION NUMBER: 2002:1084 USPATFULL

TITLE: Compositions and methods for protein secretion

INVENTOR(S): Weiner, Joel Hirsch, Edmonton, CANADA

Turner, Raymond Joseph, Calgary, CANADA

PATENT ASSIGNEE(S): University of Alberta, Alberta, CANADA (non-U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 6335178 B1 20020101

APPLICATION INFO.: US 1998-85761 19980528 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-53197, filed on 1 Apr 1998, now patented, Pat. No. US 6022952

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Cochrane Carlson, Karen

ASSISTANT EXAMINER: Mitra, Rita

LEGAL REPRESENTATIVE: Medlen & Carroll, LLP

NUMBER OF CLAIMS: 14

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 33 Drawing Figure(s); 32 Drawing Page(s)

LINE COUNT: 2762

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to compositions and methods for secretion of functional proteins in a soluble form by host cells. In particular, the invention relates to membrane targeting and translocation proteins, MttA, MttB and MttC and to variants and homologs thereof. The membrane targeting and translocation proteins are useful in targeting protein expression to the periplasm of gram negative bacteria and to extracellular media of other host cells. Such expression allows secretion of expressed proteins of interest in a functional and soluble form, thus facilitating purification and increasing the yield of functional proteins of interest.

DETD . . . integrated in a cell membrane may be solubilized (i.e., rendered into a soluble form) by treating purified inclusion bodies or cell membranes with denaturants such as guanidine hydrochloride, urea or sodium dodecyl sulfate (SDS). These denaturants must then be removed from the solubilized protein preparation to allow. . .

L46 ANSWER 51 OF 69 MEDLINE DUPLICATE 10

ACCESSION NUMBER: 92092649 MEDLINE

DOCUMENT NUMBER: 92092649 PubMed ID: 1684401

TITLE: Ultrastructural localization of scrapie prion proteins in cytoplasmic vesicles of infected cultured cells.

AUTHOR: McKinley M P; Taraboulos A; Kenaga L; Serban D; Stieber A; DeArmond S J; Prusiner S B; Gonatas N

CORPORATE SOURCE: Department of Neurology, University of California, San Francisco.

CONTRACT NUMBER: AG02132 (NIA)

AG08967 (NIA)

NS14069 (NINDS)

+

SOURCE: LABORATORY INVESTIGATION, (1991 Dec) 65 (6) 622-30.  
Journal code: 0376617. ISSN: 0023-6837.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199201

ENTRY DATE: Entered STN: 19920216

Last Updated on STN: 19950206

Entered Medline: 19920124

AB Infectious scrapie prions are composed largely, if not entirely, of an abnormal isoform of the prion protein (PrP) designated PrPSc. In scrapie-infected mouse neuroblastoma (ScN2a) and hamster brain (ScHaB) cells, PrPSc accumulates primarily within the cell cytoplasm, whereas cellular PrP (PrPC) is anchored to the external surface of the plasma membrane by a glycoinositol phospholipid moiety. To determine the subcellular localization of PrPSc, scrapie-infected cells were grown to approximately 75% confluency, fixed briefly, and then incubated with guanidine thiocyanate before antibody staining and examination by electron microscopy. PrPSc immunoreactivity was enhanced by denaturation with guanidine isothiocyanate which also permeabilized cells (Taraboulos et al., J Cell Biol 110:2117, 1990). As judged both by deposition of immunoperoxidase reaction product (diaminobenzidine) and by presence of immunogold particles, PrPSc was identified in discrete vesicular foci and some large bodies in the cytoplasm of scrapie-infected cells. Some

vesicles with PrPSc staining also contained myelin figures resembling those found in autophagic vacuoles forming secondary lysosomes. The presence of PrPSc in secondary lysosomes is inferred from colocalization of guanidine isothiocyanate enhanced PrP immunoreactivity and acid phosphatase. Neither the diaminobenzidine reaction product nor immunogold particles were observed in association with the nucleus, endoplasmic reticulum, or Golgi stacks. Exposure of scrapie-infected cells to the brefeldin A dispersed the Golgi apparatus but did not alter the morphologic distribution of PrPSc, indicating that no detectable PrPSc was associated with Golgi stacks. It remains to be established whether secondary lysosomes are involved in the post-translational formation of PrPSc.

L49 ANSWER 33 OF 41 MEDLINE

DUPLICATE 9

ACCESSION NUMBER: 95326610 MEDLINE

DOCUMENT NUMBER: 95326610 PubMed ID: 7541493

TITLE: ImmunoMax. A maximized immunohistochemical method for the retrieval and enhancement of hidden antigens.

AUTHOR: Merz H; Malisius R; Mannweiler S; Zhou R; Hartmann W; Orscheschek K; Moubayed P; Feller A C

CORPORATE SOURCE: Department of Pathology, Medical University of Lubeck, Germany.

SOURCE: LABORATORY INVESTIGATION, (1995 Jul) 73 (1) 149-56.  
Journal code: 0376617. ISSN: 0023-6837.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199508

ENTRY DATE: Entered STN: 19950822  
Last Updated on STN: 19960129  
Entered Medline: 19950810

AB BACKGROUND: Since the introduction of mAb, immunohistochemistry has become an important tool in research and in surgical pathology. The most widely used fixative in routine histopathology is formaldehyde, and it has become the gold standard for morphologic tissue preservation. Although the molecular mechanism underlying the tissue fixation is not well understood, it has become clear that available immunoreactive Ag are progressively lost during the fixation process. For a long time, it was thought that formalin-sensitive Ag might be irreversibly destroyed during the fixation process. Although monoclonal anti-Ig Ab frequently worked inadequately, polyclonal anti-Ig Ab were shown to produce reproducible staining results. It thus appeared possible that most cellular Ag might not be irreversibly destroyed but only masked. EXPERIMENTAL DESIGN: Although some Ag may be retrieved under appropriate conditions, there might still be many for which available antigenic epitopes are still too sparse to be visualized, as observed for a large number of leukocyte differentiation Ag. One reliable approach to resolve this dilemma is the use of a combination of an optimized Ag retrieval system and a powerful immunohistochemical staining protocol introducing a biotin amplification step, in which signal amplification is accomplished by covalent deposition of biotin molecules. RESULTS: Cryostat and paraffin sections were stained with the avidin-biotin complex technique and, for comparison, with the new maximized immunohistochemical staining protocol, termed the ImmunoMax method. Each step was monitored to establish how effectively it enhanced the overall sensitivity. Although pretreatment with detergent, protease, a



chaotropic substance, or microwave heating resulted in only moderately improved immunostaining, the biotinylated tyramine enhancement step proved to be the most efficient one, although the latter is not sufficient for many Ag when used without pretreatment steps. The combination of an Ag retrieval step with the biotinylated tyramine enhancement step resulted in a 100 to 10,000-fold boost in sensitivity without loss of specificity. CONCLUSIONS: With the ImmunoMax method, defined Ag can be reproducibly detected in formalin-fixed, paraffin-embedded tissues, and the sensitivity of the method is tremendously enhanced. Moreover, it also allows many previously unreactive or unsatisfactorily reactive Ag to be detected, as shown here for IgD, IgM, and CD7 with the use of mAb.

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FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 07:42:42  
ON 06 APR 2003

L1 15040 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P)  
(NONSPEC  
L2 77051 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (CELL?)  
L3 11419 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (STAIN?)  
L4 150 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3P) (TYRAMID  
L5 3696 S L1 (6P) L2  
L6 420 S L5 (6P) L3  
L7 420 S L5 (6P) L6  
L8 420 S L5 (3P) L6  
L9 5 S L1 (3P) L2 (3P) L3 (3P) L4  
L10 2 DUP REM L9 (3 DUPLICATES REMOVED)  
L11 5 S L1 (6P) L2 (6P) L3 (6P) L4  
L12 344 DUP REM L8 (76 DUPLICATES REMOVED)  
L13 1 S L12 (6P) (CHAOTROP? (6A) WASH?)  
L14 540 S CHAOTRO?/TI  
L15 1 S L12 AND L14  
L16 6203 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (NONSPECI  
L17 5432 S L2 (3P) L3  
L18 143 S L17 (3P) L16  
L19 4 S L18 (3P) L4  
L20 4 S L18 AND L4  
L21 2 DUP REM L20 (2 DUPLICATES REMOVED)  
L22 6 S L18 AND (?TYRAMINE? OR ?TYRAMIDE?)  
L23 4 DUP REM L22 (2 DUPLICATES REMOVED)  
L24 143 S L17 (P) L16  
L25 85 DUP REM L24 (58 DUPLICATES REMOVED)  
L26 17 S L4 (3P) (DENATUR?)  
L27 11 DUP REM L26 (6 DUPLICATES REMOVED)  
L28 38 S L6 (6P) (DENATUR? (P) CELL?)  
L29 38 DUP REM L28 (0 DUPLICATES REMOVED)  
L30 9872 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3A)  
(DENATUR  
L31 56002 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (CELL?)  
L32 1826 S L30 (P) L31  
L33 2 S L32 (6P) (?TYRAMI?)  
L34 16 S L32 (6P) L16  
L35 8 DUP REM L34 (8 DUPLICATES REMOVED)  
L36 1 S L32 (6P) ((NONSPECIFIC OR NON-SPECIFIC) (3A) (STAIN? OR BACK  
L37 495 S L32 (6P) (STAIN? OR BACKGROUND OR SIGNAL?)  
L38 35 S L32 (6P) (NONSPECIFIC OR NON-SPECIFIC)

L39 15 S L37 AND L38  
L40 15 DUP REM L39 (0 DUPLICATES REMOVED)  
L41 5 S L32 (6P) (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L42 41 S L32 AND (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L43 7 S L32 (12P) (BACKGROUND (3A) (SIGNAL? OR STAIN?))  
L44 7 DUP REM L43 (0 DUPLICATES REMOVED)  
L45 106 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (3A) (DENATUR  
L46 69 DUP REM L45 (37 DUPLICATES REMOVED)  
L47 64 S (CHAOTROP? OR THIOCYANATE OR GUANIDINE OR UREA) (P) (SIGNAL?  
L48 0 S L47 AND L45  
L49 41 DUP REM L47 (23 DUPLICATES REMOVED)

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